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TRANSMISSION OF OROPOUCHE VIRUS FROM MAN TO HAMSTER BY THE MIDG--ETC(U)
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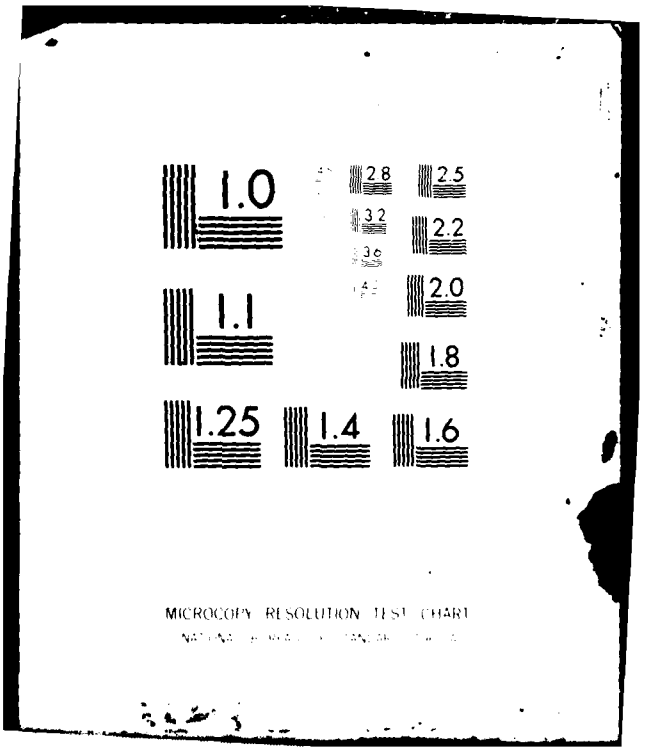
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Oropouche virus (arbovirus family Bunyaviridae, Simbu serogroup) was experimentally transmitted from man to hamster by the bite of Culicoides paraensis. Infection rates of 34% and 13% and transmission rates of 17% and 5% were observed after Culicoides engorged on patients with viremia levels of 6.3-7.3 and 5.3-6.2 $\log_{10}$ $SMLD_{50}$/ml of blood, respectively. Threshold titer necessary to enable infection or transmission by the midges was approximately 5.3 $\log_{10}$ $SMLD_{50}$/ml of blood. Transmission was achieved 6 to 12 days after C. paraensis had taken the infective blood meal. (continued)

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This represents the first conclusive evidence of transmission of an arbovirus of public health importance to man by a member of the Ceratopogonidae family.

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2 November 1981

Editor, Science
American Association for the Advancement of Science
1515 Massachusetts Ave., NW
Washington, DC 20005

Dear Sirs:

Please consider the inclosed manuscript, "Transmission of Oropouche virus from man to hamster by the midge Culicoides Paraensis," for publication in Science. The paper will be of interest to your readers under the following topics: Medicine, Virology, Entomology, Epidemiology, and Public Health. A list of six suggested reviewers familiar with these areas of investigation is attached.

Dr. Pinheiro, the first author, is now employed by the Pan American Health Organization, Washington, DC. He is, however, currently abroad, so I am submitting the manuscript in his absence. Editor's response may be directed to either Dr. Pinheiro or myself.

Thank you for your careful consideration of our manuscript.

Sincerely,

2 Incl
As stated

JAMES W. LeDUC, Ph.D.
MAJ, MS
Chief, Epidemiology Department
Medical Division

List of Suggested Reviewers

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   School of Public Health
   University of California
   Los Angeles, CA 90024

2. Dr. Thomas P. Monath
   Vector-Borne Diseases Division
   Center for Disease Control
   P.O. Box 2087
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   Department of Veterinary Science
   University of Wisconsin
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Transmission of Oropouche Virus from Man to Hamster by the Midge *Culicoides paraensis*

Francisco P. Pinheiro, James W. LeDuc, Amelia P. A. T. Rosa, Maria L. C. Gomes, Alfred L. Hoch

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

Abstract. Oropouche virus (arbovirus family Bunyaviridae, Simbu serogroup) was experimentally transmitted from man to hamster by the bite of Culicoides paraensis. Infection rates of 34% and 13% and transmission rates of 17% and 5% were observed after Culicoides engorged on patients with viremia levels of 6.3-7.3 and 5.3-6.2 $\log_{10}$ $SMLD_{50}$/ml of blood, respectively. Threshold titer necessary to enable infection or transmission by the midges was approximately 5.3 $\log_{10}$ $SMLD_{50}$/ml of blood. Transmission was achieved 6 to 12 days after C. paraensis had taken the infective blood meal.

This represents the first conclusive evidence of transmission of an arbovirus of public health importance to man by a member of the Ceratopogonidae family.

During the past two decades Oropouche (ORO) virus (arbovirus family Bunyaviridae, Simbu serogroup) has been recognized as a major cause of human febrile illness in the Amazon region of Brazil. Between 1961 and 1980 numerous outbreaks occurred in urban centers of Pará State, in the eastern part of Amazonia (1). At least 165,000 persons were infected, including 130,000 in 1978-80, when the greatest wave yet recorded affected 16 localities of this State, including the capital (2, 3). Outside of the Amazon region, human infection caused by ORO virus has been recognized only in Trinidad, where it was first isolated in 1955, but to date no epidemics have been recorded in that country (4).

Three types of clinical syndromes have been associated with ORO virus infection: (a) Febrile illness, (b) febrile illness with rash, and (c) meningitis or meningismus. Although no fatalities have been attributed to the disease, many patients become severely ill, some to the point of prostration. Acute manifestations usually last one week or less, but many patients experience one or more episodes of recurrence of symptoms for a period of one or two weeks. Instances of meningitis associated with ORO virus infection were observed during the 1980 outbreak in Pará, and while no sequelae occurred among these patients, it is clear that meningitis is an aggravating component of ORO virus infection (5). A rash also is occasionally observed on the trunks, arms and less commonly on the thighs (3).

Oropouche virus probably occurs in nature in two distinct cycles: a sylvatic cycle which is responsible for maintenance of the virus in nature, with primates, sloths and possibly certain species of wild birds implicated as vertebrate hosts, with the sylvatic vector still unknown; and an urban cycle during which man may be infected and once

infected, probably serves an an amplifying host of the virus among hematophagous insects. Two insect species have been implicated as potential vectors in the urban cycle through epidemiological studies made during outbreaks: the ceratopogonid midge Culicoides paraensis and the mosquito Culex p.quinquefasciatus (6, 7). Laboratory transmission studies of both suspect vectors found the former to be the more efficient of the two, but these experiments employed hamsters as donor and recipient host (8). Viremia titers in experimentally infected hamsters are usually much higher than those in man; consequently the question remained whether C. paraensis could become infected when feeding on the lower titered viremia which is circulated when man is infected. In this report we describe the successful transmission of ORO virus from man to hamsters by the bite of C. paraensis. This observation is especially relevant since it represents the first definitive evidence of transmission of an arbovirus pathogenic to humans by a vector of the family Ceratopogonidae.

Suspected cases of ORO virus infection which occurred during the 1979-1980 Pará outbreak were chosen for the study. Most patients were selected during their first two days of illness when viremia titers are usually highest. Blood was collected from febrile patients, diluted 1:10 in phosphate buffered saline (PBS) with 0.75% bovine albumin, and frozen at 70°C for subsequent virus titration. Viremia values were calculated by the method of Reed and Muench (9) following inoculation of serial tenfold blood dilutions intracerebrally (ic) into suckling mice. Virus identity was confirmed by complement-fixation (CF) tests using harvested mouse brains as antigen and hyperimmune mouse ascitic fluid (HMAF) prepared against the Belem ORO virus prototype strain (BeAn 19991).

All midges used in the transmission experiments were obtained as adults by man-biting collections at an agriculture research institute located near Belém where banana and cocoa trees are cultivated (8). The Culicoides were maintained at 22-25°C and 95% relative humidity and were provided with a 10% sucrose solution which was removed a few hours prior to feeding on the patients.

Shortly after initial blood collection, twenty to 100 Culicoides were allowed to engorge for about one hour on each patient's hand, usually late in the afternoon. Following feeding, midges were immobilized at 4°C and engorged specimens removed and placed in a separate holding cage and maintained with 10% sucrose solution. Unfed insects were discarded or held and later tested for the presence of virus by intracerebral inoculation into suckling mice. Five or more days after feeding on patients, attempts were made to feed the midges on laboratory-bred, newly weaned Syrian hamsters. The Culicoides were placed in small glass tubes, the open ends of which were put in contact with the shaved abdomen of a hamster. One to three insects were allowed to feed on each hamster. Following feeding, engorged insects were immediately frozen at -70°C pending virus assay. Engorged midges were later triturated, suspended in PBS containing bovine albumin and the supernatant was inoculated ic into baby mice and Vero cell cultures. Fluids from Vero cells showing cytophatic effect (3+ to 4+) were harvested and ORO virus was identified by a neutralization test using Vero cells and reference HMAF to the prototype ORO virus. Hamsters were observed daily for signs of illness. Brains and livers of morbid hamsters were removed and used as antigens in CF tests with reference HMAF for virus identification. Surviving hamsters were tested for the presence of antibody to ORO

virus by hemagglutination inhibition test 3 weeks after they had been exposed to the bites of the Culicoides.

Data obtained in transmission studies are summarized in Table 1. C. paraensis were *not* infected after feeding on patients circulating less than 5.3 $\log_{10}$ $SMLD_{50}$/ml of ORO virus. Above this apparent threshold both infection and transmission by Culicoides was clearly related to the amount of virus in donor human blood. Six of seven patients having viremia in excess of 6.2 $\log_{10}$ $SMLD_{50}$/ml infected 12 of 35 (34%) midges. Six of 12 of these Culicoides which in turn fed on hamsters transmitted the virus. Infection and transmission rates were lower when midges fed on patients having viremia of 5.3-6.2 $\log_{10}$ $SMLD_{50}$/ml. Virus was not recovered from 514 Culicoides which did not take a visible quantity of blood after exposure to 12 patients.

Although previous studies suggested that C. paraensis is the probable urban vector of ORO virus, conclusive evidence has until now been lacking. Epidemiological evidence was based mainly on the fact that a higher incidence of human infections occurred in areas with high densities of C. paraensis (7). A preplexing finding, however, has been the low isolation rates of ORO virus from C. paraensis collected during outbreaks. Only 10 isolations have been obtained from about 125,000 C. paraensis examined, a rate of approximately 1:12,500. Our findings suggest that the threshold required to enable C. paraensis to transmit ORO virus is close to 5.4-5.5 $\log_{10}$ $SMLD_{50}$/ml, a viremia titer not uncommon among ORO patients. Consequently, viremia titer alone does not appear to be responsible for the low recovery rates of ORO virus from C. paraensis.

Hamster-to-hamster transmission of ORO virus by the other suspected urban vector, Cx.p.quinquefasciatus has been accomplished (10). The threshold of infection for these mosquitoes is, however, quite high ($\geq$ 9.5 $\log_{10}$ $SMLD_{50}$/ml), well above viremia titers usually seen among ORO patients. Efficiency of virus transmission by this mosquito is low. Thus, we conclude that C. paraensis is the more important vector of ORO virus.

Culicoides have been recognized as the vectors of certain arboviruses responsible for serious diseases of domestic animals such as bluetongue, African horse sickness, and Akabane fever (11-17). Among arboviruses which cause significant human disease, eastern equine encephalitis and Congo viruses have been sporadically isolated from Culicoides (12), but these insects are not considered of importance for the maintenance of these agents.

Our findings which indicate that C. paraensis can become infected with ORO virus after feeding on viremic patients and efficiently transmit the virus to hamsters, represent the first conclusive demonstration of an arbovirus disease of man of major importance that is transmitted by Culicoides. It will be important to develop methods for the control of C. paraensis in order to prevent or interrupt epidemics. This is of particular importance in view of the increasing activity of ORO virus in urban centers of eastern Amazonia and the first report of an epidemic in the western part of this region, where the large city of Manaus was extensively affected (13). Spread of the virus to other urban centers infested with C. paraensis outside of the Amazon region (14) must also be considered.

FRANCISCO P. PINHEIRO

AMELIA P. A. TRAVASSOS DA ROSA

MARIA L. C. GOMES

Instituto Evandro Chagas

Fundacao Servicos de Saude Publica, Ministerio da Saude

CP. 621

66.000 Belem, Brasil

JAMES W. LeDUC

ALFRED L. HOCH

Department of the Army

Division of Medicine

U. S. Army Medical Research Institute of Infectious Diseases

Fort Detrick, Frederick, Maryland 21701

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Table 1. Transmission of Oropouche virus from Man to Hamster by *Culicoides paraensis*.

Viremia	No. patients exposed (No. infecting *Culicoides*)	*Culicoides* infected/ engorged (%)	*Culicoides* transmitted/ infected
6.3 - 7.3*	7 (6)	12/35 (34%)	6/12 (50%)
5.3 - 6.2	16 (5)	15/115 (13%)	6/15 (40%)
$\leq$ 5.2	4 (0)	0/31	-
TOTAL	27 (11)	27/181 (15%)	12/27 (44%)

*$\log_{10}$ $SMLD_{50}$/ml


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